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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Tai-Jay Chang
Serial No. : 09/781,693
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Title : ANDROGEN RECEPTOR COMPLEX-ASSOCIATED PROTEIN

Art Unit : 1632
Examiner : M. Pak

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION BY THE INVENTOR

I, Tai-Jay Chang, hereby declare that:

1. I am the sole inventor of the subject matter described and claimed in the above-identified application, which relates to an androgen receptor complex-associated protein or ARCAP protein.

2. I or others have conducted the following two experiments to examine the role of ARCAP protein in tumorigenesis.

3. In Experiment 1, an ARCAP cDNA was cloned into the PLXSN expression vector (BD Biosciences Clontech, Palo Alto, CA) to generate the vector, PLXSN-ARCAP. This vector was transfected into mouse liver oval WB1A cells, which do not express endogenous ARCAP protein. To obtain cells stably expressing ARCAP protein, the cells were cultured in a medium containing G418 to select cells resistant to G418. The selected cells were found to express ARCAP protein. WB1A cells stably transfected with the PLXSN backbone vector were obtained in the same manner.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

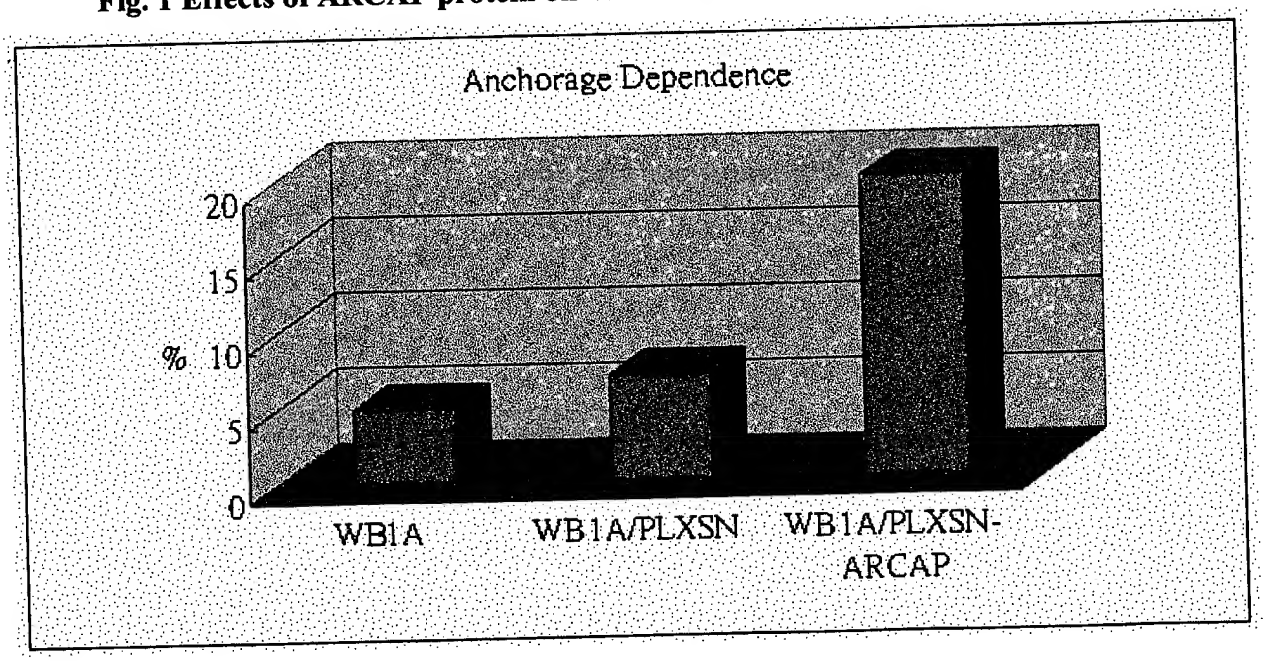
September 30, 2003
Date of Deposit

Tai-Jay Chang
Signature

TAI-JAY CHANG
Typed or Printed Name of Person Signing Certificate

Both types of transfected cells were then tested for their ability to grow anchorage-independently, which is a trait of tumorous cells. The cells stably transfected with the PLXSN-ARCAP and the PLXSN vectors ("WB1A/PLXSN-ARCAP" and "WB1A/PLXSN") were counted and seeded onto plates containing semi-soft agar, respectively. After three weeks, cell colonies were found on the top of the semi-soft agar. These colonies, each derived from a single seeded cell, contained cells growing anchorage-independently. The colonies on each plate were counted under a microscope. The percentage of the seeded cells that grew anchorage independently was calculated. WB1A cells not transfected with any vector ("WB1A") were also tested in the same manner. The results are summarized in Fig. 1 below.

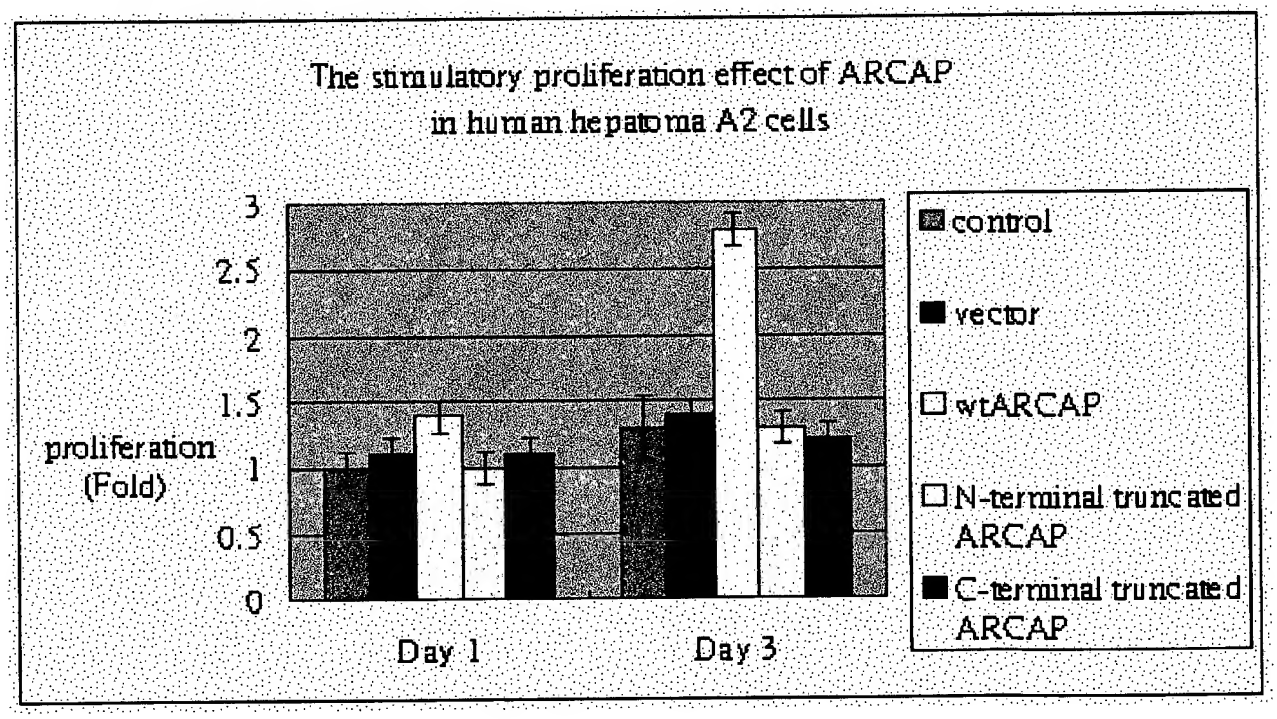
Fig. 1 Effects of ARCAP protein on WB1A cells



As shown in Fig.1, about 18% of the WB1A cells stably transfected with the PLXSN-ARCAP vector grew anchorage-independently. In contrast, only about 6% of the PLXSN transfectants grew anchorage-independently.

4. In Experiment 2, the above-mentioned PLXSN-ARCAP vector and expression vectors containing the sequences encoding a N-terminal truncated portion and a C-terminal truncated portion of ARCAP protein (amino acid 1 to 229 and amino acid 791 to 861), respectively, were transfected into hepatoma A2 cells. Cells stably expressing ARCAP protein ("wtARCAP") or the truncated portions of ARCAP protein ("N-terminal truncated ARCAP" and "C-terminal truncated ARCAP") were isolated by G418 selection as described above in Experiment 1. The selected cells were seeded onto two sets of plates containing culture media at the same density, respectively. At Days 1 and 3 after the seeding, growth of the cells on each plate set was examined using the MTT assay (Promega, Madison, WI). Cells stably transfected with the PLXSN backbone vector ("vector") and cells not transfected with any vector ("control") were also examined in the same manner. The results are shown in Fig. 2 below.

Fig. 2. Effects of ARCAP protein on the growth of hepatoma A2 cells



As shown in Fig. 2, at Day 3 after the seeding, the number of the cells stably expressing the full length ARCAP protein was twice of those of the control, the cells stably transfected with

the PLXSN backbone vector, or the cells stably expressing the truncated portions of ARCAP protein cells.

3. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,

Date: Sep. 24 2003

Tai-Jay Chang

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